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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
MOORE, WILLIAM W	

ART UNIT	PAPER NUMBER
1656	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/567,073	BRYAN, PHILIP N.	
	Examiner	Art Unit	
	WILLIAM W. MOORE	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 November 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,4,6,7,9-13 and 15-61* is/are pending in the application.

4a) Of the above claim(s) *18-45 and 50-61* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,4,6,7,11-13,16,17 and 46-49* is/are rejected.

7)☒ Claim(s) *9,10 and 15* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *20091002*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *See Continuation Sheet*.

Continuation of Attachment(s) 6). Other: Note the notice of Sequence Rules Compliance stated at page 3 of this Office communication.

DETAILED ACTION

Response to Amendment

Applicant's Amendment filed 17 November 2009 amends claims 1, 3, 4, 6, 7, 9, 10, 12, 13, and 46-48 and cancels claims 2, 5, 8, 14, and 62. Claims 1, 3, 4, 6, 7, 9-13, and 15-61 remain in the application, of which claims 18-45 and 50-61 remain withdrawn from consideration pursuant to Applicant's election of the invention of Group 1 in the reply filed 18 January 2008. Methods and products of the recently-amended claims of Group 1 now require that nucleic acid constructs comprise a nucleic acid sequence encoding a fusion protein comprising a subtilisin prodomain that is "operatively-linked" to, e.g., co-translated with, a desired protein. Several claims of Group 1 as amended further require that a subtilisin prodomain be capable of binding with a dissociation constant, "Kd", "of 10 nM or less" to either a native subtilisin or to a variant subtilisin. The amendment raises no issue of enablement or written description because the specification discloses seven variant subtilisins, i.e., the modified subtilisin BPN' species S189, S190, S196, S197, S198, S199, and S201, each of which retains binding activity for peptides that may comprise either native or modified prodomains and because other prior art subtilisin prodomains share significant amino acid sequence similarity with the subtilisin BPN' prodomain amino acid sequence of SEQ ID NO:2, thus can be modified according to the teachings of the specification. Several errors that appeared in the amendments to the specification filed 17 November 2009, however, are the bases for new objections to the specification below and a Requirement for Compliance with the Sequence Rules is also stated herein, addressing five tetrapeptide sequences occurring in the specification and/or the claims, but the standard three month, shortened statutory period for reply, with extension to six months, is permitted.

The claim amendments overcome the objection of record to the specification for lack of a statement of a Sequence Identifier in claim 10, and the objection is WITHDRAWN. Applicant's arguments together with the claim amendments overcome rejections of record of claims herein under 35 U.S.C. §§ 102(a) and 102(e) and these rejections are WITHDRAWN. Since the art recognizes that an ability to bind a cognate prodomain is one "activity" of a subtilisin and factors that contribute to retention of this activity are taught in the specification, the rejection of record of claims 3, 7, and 13 herein under the second paragraph 35 U.S.C. § 112 is overcome by the claim amendments and this rejection of record is WITHDRAWN. Rejections of record of claim 62 are rendered moot by its cancellation. As noted below, this communication is made FINAL because the claim amendments necessitate new grounds of rejection combining Ruan et al., 1999, of record, with the teachings of Ruan et al., 1998, Van Rooijen et al., and Grøn et al.

Sequence Rules Compliance

This is a Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures. This application contains five tetrapeptide sequence disclosures encompassed by the definitions for amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the following reasons: The independently-stated tetrapeptides SGIK, FKAM, FKAY, FKAF, and AHAY are not represented in the paper or computer readable forms of the Sequence Listing. In responding to this communication, Applicant is required to submit a **Revised Sequence Listing** in both paper and computer readable forms that adds the five tetrapeptides as separate sequences commencing after the current SEQ ID NO:8 and to also submit a Statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 CFR 1.821(e)-(g), 37 CFR 1.825(b), and 37 CFR 1.825 (g), In addition, these five tetrapeptide amino acid sequences must be identified by a sequence identification number wherever they occur in specification and claims. Applicant's attention is directed to 37 CFR 1.821(d), which states:

> Where the description or claims of a patent application discuss a sequence that is set forth in the "Sequence Listing" in accordance with paragraph (c) of this section, reference must be made to the sequence by use of the sequence identifier, preceded by "SEQ ID NO:" in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application.

Thus, each time that reference is made to any of the tetrapeptides SGIK, FKAM, FKAY, FKAF, and AHAY in the specification or in the claims, the reference should be accompanied by the sequence identifier, "SEQ ID NO:N", where N is an integer corresponding to the entry of the tetrapeptide sequence in the **Revised Sequence Listing**. For example, "SGIK" appears at page 20, line 33, of the specification, the tetrapeptides FKAM, FKAY, and FKAF appear at page 5, lines 29 and 28, of the specification as well as in the current claims 15, 21, 38, and 45, where the tetrapeptide FKAM appears separately in claim 9, while "AHAY" appears at page 22, line 1, of the specification.

Objection to the Specification

The disclosure is objected to because of the following informalities: In the paragraphs at page 4, lines 1-12, page 5, lines 24-30, and spanning pages 17 and 18 of the specification misstated Sequence Identifiers occur where the 30-amino acid sequence of the pre-sequence, or signal peptide, of subtilisin, is not SEQ ID NO:2 but is instead SEQ ID NO:1, while the 77-amino acid sequence of the prodomain of subtilisin is not SEQ ID NO:1, but is instead SEQ ID

NO:2. In addition, at page 25, line 8, of the specification the generic designation "Streptococcal" is misspelled. Appropriate correction is required in each instance.

Claim Objections

Claim 4 is objected to because of the following informalities: Claim 4 erroneously recites, "prodomain protein . . . variant of SEQ ID NO:1", because the 77-amino acid sequence of the prodomain of subtilisin is not SEQ ID NO:1 and is instead SEQ ID NO:2. Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 USC § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3, 4, 6, 7, 11-13, 17, and 46-49 are rejected under 35 USC § 103(a) as being unpatentable over Ruan et al., 1999, made of record with Applicant's IDS, and van Rooijen et al., US 7,531,325, in view of Grøn et al., 1996, and Grøn et al., 1992, all of record.

This is a new ground of rejection necessitated by Applicant's amendments of claims 1, 12, and 46 requiring a particular degree of binding affinity, expressed as a dissociation constant, for a prodomain of a fusion protein. Applicant's arguments at pages 14-20 of the Remarks filed 17 November 2009 have been fully considered but are moot in view of the new grounds of rejection. The specification provides no particular definitions of the terms "subtilisin activity" and "activity of subtilisin", thus these terms are construed broadly to reach any activity of a subtilisin for the purposes of this rejection, including the hydrolytic activity required by claim 47. The term "operatively linked" is broadly construed to require a peptide bond because the specification does not particularly define the phrase "operatively linked". Ruan et al., 1999, teach the design and preparation of a nucleic acid construct comprising a coding region for a variant subtilisin prodomain region, termed proR9, that possesses the binding affinity, expressed as a dissociation constant of "10nM or less" that is now required by Applicant's claim amendments of claims 1, 12, and 46 and that, among its stabilizing amino acid substitutions, comprises a substitution of lysine for the native amino acid at its P3-position, i.e., the position corresponding to position 75 within SEQ ID NO:2 herein, according to claims 4, 6, and 49 herein, that contributes to an increased binding affinity for subtilisin as compared to a subtilisin prodomain

lacking the substitution, according to claims 3 and 48 herein. See Figure 2 at page 8566 and accompanying Figure legend as well as the discussion in the paragraph spanning pages 8565-8566. Ruan et al., 1999, also teach the recombinant expression of the proR9 prodomain utilizing a *Bacillus* host cell transformed with the expression construct, and the isolation of the proR9 peptide product from the culture medium of the host cell. See Materials and Methods, left and right columns, at page 8563. Ruan et al., 1999, further teach that the isolated proR9 prodomain binds to Sbt15, a proteolytically active subtilisin, in folded and unfolded forms, as well as to Sbt70, a proteolytically-inactivated subtilisin variant. See Figures 2-7 and discussion at pages 8565 through 8570.

Ruan et al., 1999, do not, however, teach or suggest the preparation of a nucleic acid construct that will provide a variant subtilisin prodomain proR9 as an amino-proximal, or a carboxyl-proximal, fusion partner of another fusion partner, such as a peptide hormone, in a fusion protein, or the recombinant production and isolation of such a fusion protein. Thus van Rooijen et al. are now cited for teaching the preparation of polynucleotides encoding fusion polypeptides comprising any of several, diverse, polypeptide fusion partners, including hormones according to claims 11 and 16 herein, fused to modified serine protease prodomains wherein such modified prodomains have improved affinity for the cognate protease as shown by increased efficiency of production of the cleaved fusion partner, relative to the efficiency provided by an unmodified prodomain, and further teach the use of the modified prodomain as a separable component for purification of desired fusion partners, including hormones, by affinity chromatography, as well as the use of various host cells for recombinant production of fusion polypeptides, including several host cells of claim 17 herein. See columns 1-2, 6-9, and 11-17 and Figures 2, 3, and 6-12. Van Rooijen et al. demonstrate throughout that the orientation of a modified prodomain of to its fusion partner in an expressed fusion protein permits cleavage and release of the carboxyl-proximal fusion partner from the prodomain, a characteristic required by claim 47 herein.

Grøn et al., 1996, teach the use of modified peptide substrates representing the P4-P3-P2-P1 peptide of a generic prodomain to guide the preparation of amino acid substitutions at the P4 position of a subtilisin prodomain wherein a substitution introducing phenylalanine permits the modified prodomain to better bind a subtilisin protease than an unmodified prodomain, which P4 position corresponds to the fourth amino acid from a subtilisin prodomain's carboxyl-terminus, i.e., position 74 of SEQ ID NO:2, a preferred characteristic according to claims 4, 6, and 49 herein. See Results in Tables 2 and 3 at pages 108 and 110 and the accompanying discussion at pages 107-109. Grøn et al., 1992, teach that both of the commercially prominent subtilisins,

subtilisin BPN' and subtilisin 309, aka SAVINASE™, better bind an aromatic amino acid, such as phenylalanine, at the P4 position of either subtilisin prodomain, have non-specific amino acid preference at a P3 subtilisin prodomain position but accept charged positively-charged amino acids at this position, effectively bind an alanine at the P2 position of either subtilisin prodomain, and effectively bind any of alanine, phenylalanine, or leucine at the P1 of either subtilisin prodomain. See Tables II and III and the accompanying discussion at pages 6014-6016.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute a nucleic acid construct of claims 1, 3, 4, 6, 12, and 46-49 comprising a nucleic acid sequence encoding a fusion polypeptide that comprises the stabilized subtilisin BPN' prodomain proR9 of Ruan et al., 1999, which has a lysine at its P3 position, for the serine protease prodomain of van Rooijen et al. in a fusion to the amino terminus of a commercially or pharmaceutically important fusion partner, such as the hormone of van Rooijen et al., and to further modify the stabilized subtilisin BPN' proR9 prodomain according to the teachings of Grøn et al., 1996, and Grøn et al., 1992, by providing a phenylalanine substitution at the P4 position of the variant prodomain, as well as to recombinantly express such a fusion polypeptide in an *E. coli* or yeast cell host cell transfected according to the teachings of van Rooijen et al. with the nucleic acid construct in methods of claims 13, 16, and 17 herein, in order to recombinantly produce a fusion protein of claims 7 and 11 herein. This is because such an artisan would have appreciated the advantages of replacing the protease prodomain partner of van Rooijen et al. with a stabilized subtilisin BPN' proR9 prodomain modified according to the teachings of Ruan et al., 1999, that, in view of its very high affinity for either subtilisin, would permit specific and efficient capture of the fusion protein by a catalytically inactive subtilisin as well as the efficient hydrolytic release of a carboxyl-proximal fusion partner upon binding to a catalytically active subtilisin, and because both Grøn et al., 1992, and Grøn et al., 1996, teach that a substitution of phenylalanine at the P4 position of different subtilisin prodomains can provide an advantage in binding either of the mature subtilisins. Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success in practicing the claimed invention.

Conclusion

Claims 9, 10, and 15-16 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all the limitations of the base claim and any intervening claims. The subject matters of a modified subtilisin prodomain having the binding affinity required by claims 1 and 12 and that further comprises the terminal tetrapeptides of claims 9 and 15, or the terminal nonapeptide represented by SEQ ID NO:7

herein of claim 10, are free of the prior art of record because, although Ruan et al., 1999, teaches that an amino acid substitution that introduces lysine at a P3-position of the proR9 prodomain, i.e., the position corresponding to position 75 within SEQ ID NO:2 herein contributes to stabilization of the variant prodomain as well as its very high affinity for binding a mature subtilisin protease, and although both of Grøn et al., 1992 and 1996, suggest some of the prodomain carboxyl-proximal modifications of claims 4, 6, and 49 herein, the prior art does not fairly suggest the particular species of tetrapeptide sequences required by claims 9, 10, and 15 should be incorporated in a very high-affinity binding subtilisin prodomain such as the proR9 peptide of Ruan et al., 1999.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 571.272.0933 and whose FAX number is 571.273.0933. The examiner can normally be reached Monday through Friday between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's Supervisory Primary Examiner, Manjunath Rao, can be reached at 571.272.0939. The official FAX number for all communications for the organization where this application or proceeding is assigned is 571.273.8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571.272.1600.

/William W. Moore/
Examiner, Art Unit 1656

/Manjunath N. Rao /
Supervisory Patent Examiner, Art Unit 1656